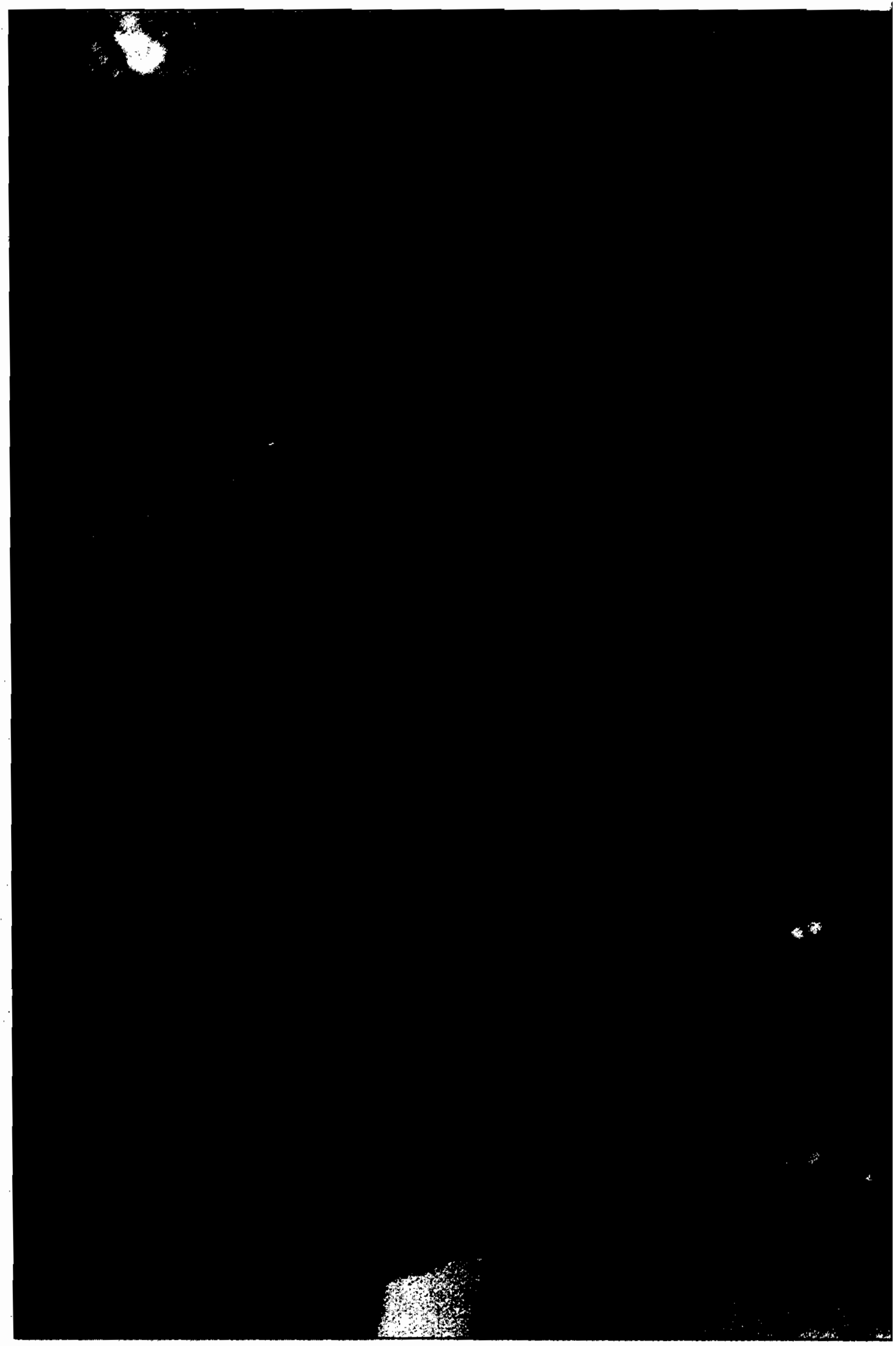
Diaprepes Root Weevil: Recent Advances at the U.S. Horticultural Research Laboratory, Ft. Pierce

By Richard T. Mayer, Wayne B. Hunter, Stephen L. Lapointe, C. L. McKenzie, R. Shatters, J. Shapiro, Hamed Doostdar and A. A. Weathersbee III

Part II: The following is Part II of a two-part series. Part I appeared in the July 2001 issue of C&VM.



Detection

Several different methods have been used to survey for adult Diaprepes root weevil (DRW) populations in groves, but none have been satisfactory for estimating both larvae and adults. Agricultural Research Service (ARS) research programs on acoustical detection of insects in grain, fruits and vegetables, have proven successful in estimating and detecting different insect populations. U.S. Horticultural Reasearch Laboratory (USHRL) scientists (Steve Lapointe, Ph.D., and Jeff Shapiro, Ph.D.) teamed with fellow ARS scientist Richard Mankin, Ph.D., (Center for Medical, Agricultural & Veterinary Entomology, Gainesville) to explore the potential of acoustics to detect hidden DRW infestations in soil and interior structures of plants. Several varieties of beetle grubs weighing 50 to 300 mg (including the scarabaeids Phyllophaga spp. and Cyclocephala spp. and the curculionids *Diaprepes abbreviatus* and *Otiorhynchus sulcatus*) were easily detected in the laboratory and in the field except under extremely windy or noisy conditions. Insect sounds could be distinguished from background noises by differences in frequency and temporal patterns, but insects of similarly sized species could not be distinguished easily from each other. Insect activity was highly variable among individuals and species, although DRW grubs tended to be more active than those of *O. sulcatus*. Tests were done to compare acoustically predicted infestations with the contents

Table 1. Oviposition per feeding bout (mean ± SE, n = 3) by DRW caged for 14 d on untreated citrus leaves in choice (one untreated and one kaolin-treated bouquet per cage) and no-choice (two untreated bouquets per cage) trials.

Treatment	Design	Eggs per cage	Eggs per bouquet
Kaolin	Choice	0.0	
	No-choice	0.0	
Untreated	Choice	104.5 ± 25.9a	104.5 ± 25.9a
	No-choice	350.9 ± 71.5b	175.4 ± 30.0a

of soil samples taken at recording sites. At laboratory or ideal field conditions, active insects within ~ 30 cm were identified with nearly 100 percent reliability. In field tests under adverse conditions, the reliability decreased to 75 percent. These results indicate that acoustic systems with vibration sensors have considerable potential as activity monitors in the laboratory and as field tools for rapid, nondestructive scouting and mapping of soil insects.

Another approach to detection stems from research by Mayer and Doostdar on host plant responses to feeding by DRW larvae. Feeding by DRW larvae induces two to three different proteins in many varieties of citrus. The proteins were isolated from Swingle citrumelo roots and found to be chitinases (enzymes that digest chitin in insects, fungi, yeasts, etc.). Antibodies were developed for the proteins and were used to determine if DRW feeding could be detected in roots and leaves. Indeed, the antibodies can be used to detect feeding in the roots and leaves, but only for some varieties of citrus.

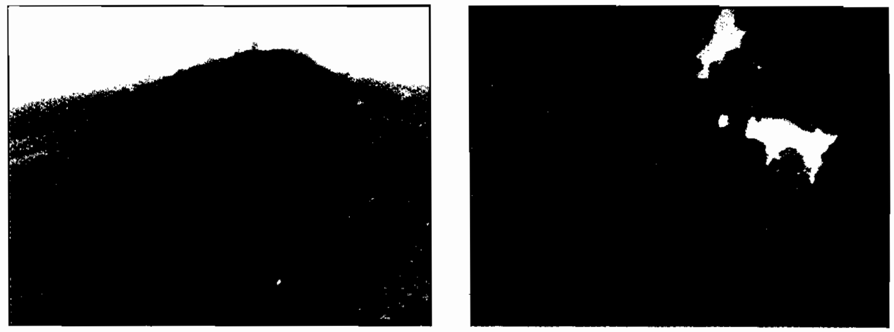
Physical Control

Particle films. The DRW is categorized as a broad-nosed weevil. Females of this group place their eggs with an adhesive in a single layer in a niche most often fabricated by juxtaposing leaves. Recent interest in particle films for control of plant pathogens and insect pests has led to the development of experimental formulations of kaolin, an inert silicate, for foliar applications. A hydrophilic formulation of kaolin was tested in a screenhouse for its effect on the behavior of DRW. Lapointe has shown that feeding by adults on treated foliage was reduced by 75 to 84 percent compared with adults fed untreated foliage. No direct insecticidal activity was detected. Egg-laying (oviposition) was completely suppressed on treated foliage (see Table 1). While females laid more than 19,000 eggs during two trials on untreated foliage, no egg masses were found on foliage treated with the kaolin formulation. This indicates potential for kaolin as a barrier to oviposition in citrus groves and may prove to be an economically viable and environmentally sound component of an integrated approach to control of DRW, related root weevils, and other insect pests such as psyllids and aphids. Additional advantage comes from increased growth of citrus trees treated with kaolin films. Increased reflection of the light decreases leaf temperature and photosynthesis may be increased to the interior of the tree canopy.

Barriers to neonate penetration. Recent work by McKenzie and Lapointe has identified two commercially available weed cloths (landscape fabric) that are effective barriers to penetration by neonate DRW larvae as they hatch, fall from the tree, and attempt to locate roots in the soil. Installation costs for such fabric may be high, however, the costs may be recouped by reduction in herbicide use and the fact that some of the fabrics can last for 10 to 20 years. This concept is being developed in field trials.

Host Plant Resistance

Identification of resistance. There have been several assessments of DRW resistance in citrus varieties over the years. Because various evaluation methods may take up to a year and are toilsome, Lapointe compared three methods for assessing resistance, including a new method that uses young (three to four-month-old) seedlings in plastic cell pots. The new method, in addition to being faster, allows for simultaneous screening of the large numbers of seedlings typically generated by the USHRL citrus-breeding program. The rapid screen was tested on a selection of cultivars, new hybrids and related citroid species. Results were comparable to reports obtained previously by more laborious methods and established that some resistance was present in Swingle citrumelo rootstock. In addition, a new source of resistance to DRW was identified in the remote citroid fruit tree, *Murraya koenigii*. This evaluation method will now be applied in a more systematic search for resistance to this important pest in citrus fruit trees.



DRW larvae uninfected (left) with isolate #39 and uninfected (right). The infected larvae is dead.

Roots of a citrus relative, *Glycosmis pentaphylla* (orangeberry), were shown by Shapiro, Bowman and Lapointe to inhibit the growth and survival of larvae of DRW. Roots of *G. pentaphylla* incorporated into the diet of DRW increasingly inhibited the growth of neonate larvae with increased concentration of roots, while roots from citrus rootstocks produced little inhibition. The diet-incorporation assay was used to guide fractionation of an active acetone extract of *G. pentaphylla* roots. The active component was identified as dehydrothalebanin B.

Experiments by Mayer, Shapiro and Doostdar on plant responses to DRW larval feeding on roots showed that basic chitinases were induced in several varieties (Swingle, Flying Dragon x Nakon, *Citrus macrophylla*, sour orange, pummelo 4N, and Cleopatra). Chitinases digest chitin, which is a structural carbohydrate polymer that comprises arthropod exoskeletons and the cell walls of fungi and yeast. Chitin is also found in the peritrophic membrane that lines the gut of many insects including DRW; the peritrophic membrane protects the digestive tract from infection by microbials and abrasion by ingested food. Cleopatra and Swingle chitinases possess good abilities to digest DRW peritrophic membranes and increased amounts of chitinases in damaged roots should cause more damage to the peritrophic membrane. The addition of bacterial chitinases to microbial biological control programs have increased efficacy by tenfold; chitinases have been suggested to create holes in the peritrophic membrane making passage of the microbe easier.

Also, the presence of these chitinases in citrus are correlated with resistance to DRW and Phytophthora. Purification and characterization of one of the induced chitinases showed that it was related to a class of anti-phytophthora proteins. DRW larval feeding is know to predispose plants to Phytophthora infections. The gene controlling the anti-phytophthora protein has now been cloned and experiments are in progress to determine if insertion of the gene into non-resistant plants will either prevent or decrease Phytopthora infections. Tests with the purified anti-phytophthora protein indicate that it acts on a number of different types of Phytophthora.

Biotechnological approaches. The use of natural biomolecules that reduce or eliminate DRW feeding on citrus can be facilitated through the development of transgenic plants containing novel genetic material or increasing number of native genes that produce the desired biomolecules. However, two major limitations to the use of transgenic crops for reducing insect damage to plants are: 1) the time required to develop a transgenic plant and/or plant product to the point of release to the grower/consumer; and 2) the practical limitations on the development of a broad germplasm base containing the desired transgenically introduced gene. These problems are exacerbated in woody perennial crops that are not planted annually. For example, the average life of a producing citrus tree is probably 20 to 25 years in a commercial setting and removal of existing trees and their replacement with resistant trees would not be economically feasible. USHRL explored the potential of using a surrogate transformation system to address these problems in citrus, i.e., the use of endophytic bacteria (endophytes) living within a citrus tree that can function as vehicles to produce and deliver desired biological products. Many of these bacteria have no pathogenic symptoms on the host plant and, in fact, some endophytes improve the fitness of the host and have been shown to be effective as biocontrol agents against pathogenic microorganisms.

Bob Shatters, Ph.D., isolated and identified 72 endophytic bacteria from 14 different citrus varieties located in Ft. Pierce. The bacteria were isolated from new-growth stem tissue. Bacterial isolates were identified by fatty acid methyl ester (FAME) analyses. Isolates were shown to represent a diverse group of bacteria with members from 14 different genera. Bacteria identified include the following: *Acinetobacter baumannii, Bacillus cereus, B. longisporus, B. lentimorbus, B. coagulans, B. oleronius, B. pumilus, B. subtilis, Brevibacillus brevis, Clavibacter michiganense, Curtobacterium flaccumfaciens, Curtobacterium pusillum, Enterobacter cancerogenus, E. agglomerans, Kluyvera cryocrescens, Microbacterium saperdae, Paenibacilus macerans, Pseudomonas aeruginosa, Pseudomonas putida, Staphylococcus haemolyticus, Staphylococcus epidermidis, Staphylococcus wereri, Stenotropomonas maltophilia.* Experiments are underway to determine how well these move through the vascular system of the plants. Gene introduction methods are being developed for the most promising microbes. The advantage of using these naturally occurring bacteria to deliver biomolecules is that they can be applied to trees in producing groves, thereby circumventing the need to remove trees and replace them with improved citrus cultivars.

Interestingly, several of the bacterial isolates did show toxic effects when applied directly to Diaprepes larvae. These are being studied for their direct use in control of Diaprepes larvae.

Biological Control

Parasitoids. A collaborative effort (ARS, the University of Florida and the U.S. Sugar Corporation) was formed to discover parasitoids of DRW eggs in Florida citrus groves infested by DRW during 1997, 1998 and 1999. The research was conducted at nine locations in Florida including the following counties: Hillsborough, Polk, Orange, Glades, Hendry, St Lucie, Indian River and Dade. One location was studied on Puerto Rico. No native parasitoids attacking DRW eggs in citrus were discovered in Florida. In contrast, an average of 33.4 percent (range 12.9 to 68.8 percent) parasitism of weevil egg masses was observed in citrus sampled on Puerto Rico. *Quadrasticus haitiensis* and Horismenus spp. were recovered from egg masses of *D. abbreviatus* in Puerto Rico. The Horismenus parasitoids are suspect hyperparasites. Releases of the parasitoid *Ceratogramma etiennei* from Guadeloupe were made during 1998 at each of the Florida research sites.

Bacterial entomopathogens. The bacterium, *Bacillus thuringiensis,* produces one or more insect toxic proteins, crystalline δ-endotoxins. Upon ingestion, these endotoxins are proteolytically activated in the midgut of susceptible insect hosts. Endotoxin activation leads to feeding inhibition, septicemia, and eventual death of the insect. Endotoxin complexes produced by *B. thuringiensis subsp. tenebrionis (Btt)* are being evaluated by Allen Weathersbee, Ph.D., against DRW larvae. Survival of hatchling larvae is reduced after exposure to either insect diet or potted citrus treated with *Btt*, but the effect is variable. Larvae exposed at five weeks of age to *Btt*-treated diet show a strong, dose-dependent mortality response but mortality was delayed for approximately 80 days. The lethal concentration for a 50 percent kill (LC_{50}) for larvae in this age group was 6.2 ppm. The delay in mortality is attributed to activation of endotoxin by proteases (e.g., trypsin) only during particular stages of larval development. The results indicate that *Btt* may have utility in pest management programs directed against DRW. A search for naturally occurring pathogenic strains of *B. thuringiensis* was initiated and a total of 266 bacterial isolates were selected from samples of soil and diseased weevil larvae collected from Florida citrus groves. To date, Weathersbee and co-workers have demonstrated the presence of at least six different classes of Cry protein (endotoxin) genes in DNA extractions from the stored isolates. Selected isolates are being evaluated for novelty and pathogenicity toward DRW.

Fungal entomopathogens. In a joint effort between the USHRL and Morse Enterprises Ltd. Inc., several entomopathogens from field collected egg masses and diseased larvae were discovered. In all, 36 fungi were recovered and subsequently evaluated by Doostdar and Mayer for their abilities to infect DRW egg masses and larvae. Further tests were executed to determine the ability of the fungi to grow rapidly on DRW egg masses and larvae, and for ease of production of spores in liquid culture. Seven fungal entomopathogens were selected and identified. These seven entomopathogens were deposited in the ARS Patent Culture Collection, Peoria, Ill; three of the filed pathogens are Fusarium species while the remainder are Bipolaris sp., Cladosporium sp., Aspergillus, sp., Alternaria sp. It is interesting that the Bipolaris sp. has never before been observed as an entomopathogen.

A U.S. patent was been applied for these seven candidate biological control agents last year. Testing continues with the fungi to determine optimum conditions for application, growing of the pathogens, formulation, etc. in the lab and in the field.

Viral Entomopathogens. Two viral insect pathogens were discovered by Hunter and Lapointe that infect all stages of DRW. Both are members of the Iridoviridae. IIV6 (Invertebrate Iridescent Virus-6) has been shown to infect eggs, larvae and adults of DRW (see figure). Transovarial (vertical) transmission (females transmit the virus to the eggs) has been demonstrated. Mechanisms of horizontal transmission (i.e., transmission between adults) are being studied at this time. A second, undescribed iridovirus is also being studied.

Diaprepes Root Weevil Emergence

The University of Florida and six grower/cooperators are conducting a year-long survey to determine the weekly emergence patterns for Diaprepes root weevils. Surveys are being conducted in Lake, Polk, Indian River, Desoto, Hendry and Dade counties. At each location, 100 Tedder's traps are surveyed weekly to determine the number of weevils collected in the traps. From this data, graphs are being developed to provide growers with average number of weevils per traps as well as total weevils collected during the weekly intervals. Growers can then determine when the best time is to apply sprays.

The information collected from the survey is available at www.lal.ufl.edu. At this site, growers can choose the county location which is closest to their grove to estimate the emergence pattern that represents their area. Other information related to Diaprepes root weevils and its control is also available at www.lal.ufl.edu.

In conclusion, the USHRL, and in particular the Subtropical Insects Research Unit, has several promising control methods under investigation for one of Florida's most serious pests to citrus. These projects have incorporated the latest science technologies to follow and evaluate leads for new control strategies for DRW. Barring any major problems, some of these methods should be available to growers within the near future. The USHRL Subtropical Insects Research Unit cooperates with UF, the Florida Division of Plant Industry, various industries, and other agencies and organizations coordinated by the Diaprepes Task Force. *CVM*

Richard T. Mayer, Wayne B. Hunter, Stephen L. Lapointe, C.L. McKenzie, R. Shatters and J. Shapiro are presently located at USDA, Gainesville, FL. Hamed Doostdar and A. A. Weathersbee III are with USDA, ARS, U.S. Horticultural Research Laboratory, Ft. Pierce, FL.